REMARKS

The present invention includes a method of inducing and enhancing the proliferation of human marrow stromal cells. Claims 1 through 41 are pending in the present application. Claims 30 and 37-41 have been withdrawn without prejudice to their inclusion in any later filed applications. Claims 1-29 and 31-36 are currently under examination.

Applicants are pleased to note that the Amendment filed December 4, 2002 has been entered and that Applicants' request for Revocation and Power of Attorney has been acknowledged and entered. Applicants are very pleased to note that the Examiner has withdrawn his rejections pursuant to 35 U.S.C. §112, second paragraph. Additionally, Applicants are very pleased to note that the Examiner has withdrawn his rejections pursuant to 35 U.S.C. §102(a) as being anticipated by DiGirolamo et al. (1999, British Journal of Haematology, 107: 275-281).

Applicants respectfully request a telephone interview between Examiner Ram R. Shukla, Ph.D. and the undersigned, Kathryn Doyle, Ph.D., J.D. in order to discuss the claims on their merits. Applicants counsel will contact the Examiner via telephone in the near future to arrange a mutually convenient time for both parties to discuss the claims on their merits.

Rejection of Claims 1-21 pursuant to 35 U.S.C. §102(b)

The Examiner has maintained his rejection of claims 1-21 under 35 U.S.C. §102(b). Specifically, the Examiner asserts that Bruder et al. (1997, Journal of Cellular Biochemistry 64: 278-294) teaches isolated bone marrow cells that have approximately 1 marrow stromal cell in 10^5 cells, which represents 100 cells in a 60 cm^2 dish. The Examiner is of the opinion that Bruder et al. meets the limitations of the claimed invention.

In order to anticipate, a prior art reference must disclose each and every limitation of the claimed invention, the reference must be enabling, and the reference must describe the claimed invention sufficiently to place it in possession of a person of ordinary skill in the art of the field of the invention. *Helifix Limited v. Blok-Lok, Ltd.*, 208 F.3d 1339 at 1346 (Fed. Cir. 2000). Bruder et al. describes plating an hMSC-enriched low density fraction at 10^7 cells per 60 cm^2 and culturing the cells. Bruder et al. estimates that the initial density of adherent hMSCs is approximately 1 in 10^5 in the total cell population. The non-adherent cells are removed, and the adherent cells were grown to confluence. This step, as described by Bruder et al. is not a method of inducing or enhancing the proliferation of MSCs, but is rather the art-accepted method of isolating adherent MSCs from non-adherent cells in a bone-marrow aspirate. This does not read

on the claimed invention because it is not a method of inducing proliferation in isolated cells; it is simply a method of isolating adherent cells from non-adherent cells. Moreover, the term "isolated" as recited in the present claims and as it is defined in the specification (page 18, line 5), describes a cell that has been substantially separated from similar entities. Bruder et al. describe MSCs that are estimated to comprise 1 cell for every 100,000 other bone marrow aspirate cells. These are not isolated cells, as defined in the instant specification and as claimed, and therefore Bruder et al. does not anticipate the present invention.

Once Bruder et al. teach how to isolate MSCs, but not how to induce or enhance proliferation, the MSCs are plated (Bruder et al., page 280, second column). Assuming that the MSCs are isolated, as defined in the specification, from other bone marrow aspirates, the cells are plated at a density of 3×10^3 cells per cm^2. This is not less than about 50 cells per cm^2, as recited in claim 1 and the claims that depend from it, and therefore, does not anticipate the present invention because Bruder et al. does not disclose every limitation of the instant claims.

As recited in claim 12 of the instant application, the isolated cells plated at an initial density of less than about 50 cells per square centimeter are provided to a second growth surface at an initial density of less than about 50 cells per square centimeter. In contrast, Figure 1 in Bruder et al. teach that the lowest density of cells used in serial passages is 3×10^3 cells per cm^2, which is not less than about 50 cells per cm^2, and does therefore not anticipate the present invention.

Applicants respectfully submit that Bruder et al. does not teach plating isolated MSCs at an initial density of less than about 50 cells per cm^2, does not disclose each and every limitation of the claimed invention, and therefore does not anticipate the claimed invention. Reconsideration and withdrawal of the Examiner's rejection pursuant to 35 U.S.C. §102(b) is respectfully requested at this time.

Rejection of Claims 1-21 pursuant to 35 U.S.C. §102(b)

The Examiner has maintained his rejection of Claims 1-21 under 35 U.S.C. §102(b). Specifically, the Examiner argues that Kuznetsov et al. (Journal of Bone and Mineral Research 12: 1335-1347) teaches single colony derived strains of human marrow fibroblasts (HMSFs) plated at $0.14\text{-}14 \times 10^3$ cells/cm^2 or $.007\text{-}3.5 \times 10^3$ cells/cm^2, and that such teachings anticipate the present invention.

Kuznetsov et al. describe plating single-colony derived HMSF strains at initial densities of 0.14-14.0 X 10^3 nucleated cells per square centimeter for aspirate derived cells and 0.007-3.5 X 10^3 nucleated cells per square centimeter for surgical specimens. In addition, the aspirate cells were plated at a higher density because of the presence of peripheral blood cells (page 1336, column 2). Thus, the plated aspirate cells were not isolated cells, as defined in the specification at page 18, line 5 and as presently claimed. In fact, according to the teachings of Kuznetsov et al., the marrow cells are obtained from either bone marrow aspirate or scraping surgical specimens. The cells are then centrifuged and passed through various needles and a cell strainer to break up aggregates. Nowhere does Kuznetsov et al. describe any process to isolate marrow stromal cells from the rest of the bone marrow cellular milieu. As described in the specification, bone marrow contains various types of multipotential cells (page 1, line 20), and the process for isolating MSC from the remainder of the cells comprising bone marrow involves density-centrifugation to remove mononuclear cells and the ability of MSCs to adhere to plastic in order to remove non-adherent cells (page 29, beginning at line 8). Kuznetsov et al. does not describe any of the art-accepted procedures for isolating MSCs from other cells, but only describes simple centrifugation and methods for breaking up cell aggregates. Kuznetsov et al. does not teach the necessary steps for isolating MSCs from other bone marrow cells, and only teaches plating a heterogeneous population of cells, therefore, Kuznetsov et al. does not teach isolated MSCs, as defined in the specification (page 18, line 5) and as presently claimed.

The initial plating density described in Kuznetsov et al. was from 140 to 14,000 cells per cm^2, and the cells were not isolated MSCs because they were contaminated with peripheral blood cells. Therefore, Kuznetsov et al. does not disclose each and every limitation of the claimed invention recited in claims 1-21, and does not anticipate the invention for the purposes of 35 U.S.C. §102(b).

Kuznetsov et al. describes plating 0.007-3.5 X 10^3 per cm^2 HMSF nucleated cells from surgical specimens. This translates to 7 to 3500 cells per cm^2. The lower density range is less than about 50 cells per square centimeter and the upper density range is greater than about 50 cells per square centimeter as presently claimed, and the upper range taught by Kuznetsov et al. does not anticipate the present invention.

As set forth above, Kuznetsov et al. does not teach isolated cells, and does not disclose each and every limitation of the claimed invention. Kuznetsov et al. further fails to

anticipate the method presently claimed because Kuznetsov et al. does not teach the method set forth in dependent claim 12, and the claims depending therefrom. As recited in claim 12, cells initially plated at a density of less than about 50 cells per square centimeter are harvested and provided to a second growth surface at a density of less than about 50 cells per square centimeter. Further, as recited in claim 16 and the claims that depend therefrom, the cells are harvested from a second growth surface and provided to a third growth surface at density of less than about 50 cells per square centimeter. Kuznetsov et al. only teaches that the individual colonies are harvested by one of two methods and plated on six-well plates. The number of cells in the second passage is not disclosed, and the area in square centimeters of the six well plates is not disclosed either. The skilled artisan would not be able, when armed with the teaching of Kuznetsov et al., to plate the harvested cells at a specific density of less than about 50 cells per centimeter squared as described in claim 12 and the claims depending therefrom. Therefore, Kuznetsov et al. does not describe the claimed invention sufficiently to place it in the possession of one of ordinary skill in the art, and therefore does not anticipate the present invention. *Helifix Limited*, Id.

In addition, Kuznetsov et al., on page 1339, second column, describes the characteristics of the plated HMSF cells. The cells are described as having three distinct populations as determined by FASCcan analysis, as well as differing characteristics as far as α-naphthyl acetate esterase staining and the presence of acid phosphatase. In fact, figure 2A on page 1340 depicts the heterogeneous population of cells, and Kuznetsov et al. describe these additional cells as macrophages and endothelial cells. Therefore, Kuznetsov et al. does not teach an isolated population of MSCs, as defined in the specification on page 18, line 5, and because Kuznetsov et al. does not disclose each and every limitation of the claimed invention, Kuznetsov et al. does not anticipate the present invention under 35 U.S.C. §102(b).

Applicants, in view of the foregoing arguments, respectfully request reconsideration and withdrawal of the rejection of claims 1-21 pursuant to 35 U.S.C. §102(b) as being anticipated by Kuznetsov et al.

Rejection of Claims 1 and 22-23 pursuant to 35 U.S.C. §103(a)

The Examiner has maintained his rejection of claims 1 and 22-23 under 35 U.S.C. §103(a) as being unpatentable over Kuznetsov et al. in view of Azizi et al. (1998, Proc. Nat'l.

Acad. Sci. USA 95: 3908-3913).

The three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

None of these criteria have been met here.

The present invention encompasses a method of inducing and enhancing the proliferation of human marrow stromal cells *in vitro* by plating the isolated cells at very low densities in the presence of a growth medium, harvesting the cells, and providing the cells to a second growth surface at a certain density of cells in the presence of growth medium. As amply detailed in the specification, such as Example 2, beginning at page 36, the methods presently claimed result in a dramatic increase in the number of population doublings without differentiation. Neither Kuznetsov et al. or Azizi et al. suggest to or motivate the skilled artisan to arrive at the present invention. That is, neither Kuznetsov et al. or Azizi et al. suggest that when isolated MSCs are plated at very low densities, they are induced to rapidly differentiate and increase in number at levels unheralded in the prior art.

Applicants argue that Kuznetsov et al. offers no suggestion or motivation to modify the reference or to combine reference teachings to arrive at the present invention. Further, Kuznetsov et al. does not offer the skilled artisan a reasonable expectation of success, and does not teach or suggest all of the claim limitations.

Kuznetsov et al. teach that plating MSFs at low densities was performed in order to prevent cross-contamination. Kuznetsov et al. refers to the plating densities as "extreme precautions" to prevent colony cross-contamination, and does not suggest that plating cells at a low density results in the induction of proliferation. Additional precautions taken to prevent cross-contamination include careful selection of colonies and avoiding mechanical disturbance of colonies (page 1343, second column). There is no suggestion that these measures were undertaken in order to induce proliferation in the MSFs, and there is no motivation to use these

very low densities to increase the proliferative capacities of MSFs. The skilled artisan, when equipped with Kuznetsov et al. would only find that the reference suggests low plating densities to ensure adequate separation between colonies so their clonal nature can be retained.
In addition, Kuznetsov et al. does not teach that increasing proliferation of MSFs would be a desirable trait, and therefore there is no suggestion to combine the teachings of Kuznetsov et al. with Azizi et al. In contrast, Kuznetsov et al. employed "extreme precautions" to prevent cross-contamination of different colonies, and therefore the addition of PDGF-AA, which, according to Azizi et al., increases cell growth, would increase the likelihood that colonies would grow into each other and result in cross-contamination. The skilled artisan, following the teachings of Kuznetsov et al., would actually find that the methods of Azizi et al. would thwart any attempt to develop isolated, clonally pure colonies of MSFs. Thus, there is no suggestion or motivation, either in the references or in the art, that would suggest combining the teachings of Kuznetsov et al. and Azizi et al.

Moreover, Kuznetsov et al. teach plating un-isolated bone marrow cells at various densities from 7 cells per cm^2 to 14,000 cells per cm^2. Nowhere does Kuznetsov et al. suggest or motivate the skilled artisan to believe that plating at various densities leads to the induction of proliferation in MSC cells. Kuznetsov et al. only describes the proliferative capacity of one group of cells, as detailed in the Results section, page 1338, second column to page 1339, first column, and Figure 1 (page 1339). Cells plated at .14 X 10^3 cm^2 (140 cells per cm^2) are depicted and described as proliferating over a period of 14 days. However, Kuznetsov et al. does not motivate the skilled artisan to plate the cells at a lower density to increase proliferation. In contrast, Kuznetsov et al. describe the rapid proliferation of cells plated at 140 cells per cm^2, and no suggestion is made to indicate that decreasing the initial density of the cells could influence the rate of proliferation or induce the cells to further proliferate. Kuznetsov et al. does not suggest that plating the cells at a lower density, such as the densities presently claimed, would increase the proliferation of these cells and thus, Kuznetsov et al. does not meet the first criteria to render the present invention obvious.

Azizi et al. teach the removal of bone marrow aspirate, the initial isolation step comprising density gradient centrifugation, and the secondary isolation step comprising differentiating MSCs from other bone marrow cells by their adherence to a plastic substrate. Azizi et al. teach that bone marrow aspirates, before they are separated from non-adherent cells,

are plated at a density of 3 X 10^6 cells per cm^2. Applicants note that these cells are not isolated from non-adherent cells that make up a portion of the bone marrow. After non-adherent cells are removed, the adherent cells are grown to confluence, split at a ratio of 1:2 or 1:3, and replated in the presence of PDGF-AA. Azizi et al. suggest that the addition of PDGF-AA increases the growth rate of the cells, but no there is no suggestion that decreasing the initial density of isolated MSCs or decreasing the density of harvested MSCs provided to a second growth surface would further induce proliferation. In fact, the only suggestion made in order to increase the number of MSCs for the experiments described in Azizi et al. is to add PDGF-AA to the media, not to decrease the initial or secondary plating density. Therefore, Azizi et al., even in view of Kuznetsov et al., fail to offer a suggestion or motivation to modify the reference to arrive at the instant invention.

The second criteria for establishing a *prima facie* case of obviousness is that there must be a reasonable expectation of success. Kuznetsov et al. only describe the proliferative and morphology characteristics of MSCs plated at a density of 140 cells per cm^2. The growth characteristics and morphology of cells plated at a lower density are not described. Thus, from the disclosure set forth in Kuznetsov et al., the skilled artisan would have not reason to expect an increase in the proliferation of cells plated at a density less than 140 cells per cm^2. The only reason Kuznetsov et al. provides for plating MSCs at low densities is that such densities were used to prevent colony cross contamination (page 1343, second column). From this, one of skill in the art has no reason to expect success in inducing proliferation of MSCs by plating the cells at very low densities other than to prevent contamination, and therefore, Kuznetsov et al. fail to render the present invention obvious.

Similarly, Kuznetsov et al. in view of Azizi et al. does not offer one of skill in the art a reasonable expectation of success in inducing isolated MSCs to proliferate. Azizi et al. teach that the addition of PDGF-AA increases the growth rate of cells, but when combined with Kuznetsov et al., there is no expectation that plating the cells at a very low density and adding PDGF-AA to the medium will result in the induction of proliferation. Because Kuznetsov et al. only teach that low plating densities are useful for preventing cross contamination between cell lines, one of skill in the art has no reasonable expectation of success in combining the teaching of the two references.

The third prong in establishing a *prima facie* case of obviousness requires the

prior art reference or references to teach or suggest all of the claim limitations. As detailed elsewhere herein, Kuznetsov et al. does not teach plating isolated cells, as defined in the specification, on a growth surface or a method for inducing proliferation of isolated cells. The cells described in Kuznetsov et al. are not isolated in the art-accepted manner, and do not seem to be isolated from the rest of the bone marrow cells in any manner. Therefore, Kuznetsov et al. do not teach or suggest all of the claim limitations. The methods disclosed in Azizi et al. are unable to correct this defect in the teachings of Azizi et al. and therefore, Kuznetsov et al. in view of Azizi et al. do not render the present invention obvious.

Applicants respectfully submit that Kuznetsov et al. in view of Azizi et al. do not render the present invention obvious, and request reconsideration and withdrawal of the Examiner's rejection pursuant to 35 U.S.C. §103(a).

Rejection of Claims 1 and 22-23 pursuant to 35 U.S.C. §103(a)

The Examiner has maintained his rejection of claims 1, 24-29 and 31-36 under 35 U.S.C. §103(a) as being unpatentable over Kuznetsov et al. in view of Azizi et al. and in further view of Greenberger et al (U.S. Patent No 5,766,950) and Prockop (1997, Science 276: 71-74). Specifically, the Examiner is of the opinion that it would have been obvious for one of skill in the art to combine the culture conditions of Kuznetsov et al. with the addition of PDGF-AA taught in Azizi et al. as well as the conditioned medium taught by Greenberger et al. and the general properties of MSCs taught by Prockop et al.

As detailed above, Kuznetsov et al. and Azizi et al. do not render the present invention obvious because the skilled artisan, when armed with the teachings of Kuznetsov et al. would not be motivated to combine those teachings with Azizi et al. because the combination of the two would result in cross-contamination of clonally segregated cell populations. Thus, not only do the teachings of Azizi et al. directly contradict the goals of the teachings set forth in Kuznetsov et al., they fail to render the present invention obvious.

Kuznetsov et al. in view of Azizi et al., Greenberger et al., and Prockop similarly fail to render claim 31 obvious because the skilled artisan would find no suggestion or motivation to isolate cells from bone marrow, incubate the cells to yield colonies, isolate an individual colony, and incubate the cells in a growth medium at a density of less than about 50 cells per square centimeter in order to induce proliferation of human marrow stromal cells. Azizi

et al. and Greenberger et al. teach cells at an initial density far greater than less than about 50 cells per square centimeter, and Kuznetsov et al. do not teach isolated cells, and the reference does not suggest that an initial density of less than about 50 cells per square centimeter would result in the induction of proliferation, but rather suggests that a low initial plating density is useful for keeping clonally pure colonies. Further, the fact that Kuznetsov et al. do not teach the isolated cells, as presently claimed and defined in the specification, the combination of references fails to teach or suggest all of claim limitations.

The Examiner has cited Figure 1 in Azizi et al. as rendering obvious claims 32-36 where a method for assessing the expandability of human marrow stromal cells is recited. Applicants note that Azizi et al. do not teach or suggest all of the claim limitations of claims 32-36 because Azizi et al. teach plating the MSCs at an initial density of 3×10^6 cells per cm^2, which is not less than about 50 cells per cm^2 as recited in claim 32. Further, as recited in claim 33, the cells are incubated for at least about 10 days. Figure 1 in Azizi et al. only shows the results of the assay for 4 days, and does not suggest that a longer incubation period would have an increased effect on the growth of the cells. Therefore, Azizi et al. do not render claims 32-36 obvious.

Greenberger et al. and Prockop similarly fail to render claims 24-29 obvious in view of Kuznetsov et al. and Azizi et al. Greenberger et al. teach a substantially different conditioned media and method for expanding stromal cells, and the skilled artisan would have no reasonable expectation of success in applying the teachings of Greenberger et al. to Kuznetsov et al. and Azizi et al. to arrive at the present invention. Greenberger et al. teach that the conditioned medium is derived from the medium of the first growth surface, centrifuged to pellet non-adherent cells, and then the cells were re-suspended in the media and returned to the first growth surface. Claim 24, and the claims that depend therefrom recite that the conditioned media is derived from a second growth surface, not from the first growth surface. Hence, the difference between the source and content (e.g. non-adherent cells from the same growth surface taught in Greenberger et al. versus the present invention) would lead to unpredictability, and one of skill in the art would have no expectation of success in combining the teachings to arrive at the present invention.

In addition, Greenberger et al. teach, at column 6, lines 30-39, the five "key" steps in the disclosed cell culture regimen. The first is to coat the growth surface with gelatin, the

second is to return non-adherent cells to the growth surface, the third is to add medium that contains sufficient nutrients, the fourth is to supplement the medium with acidic FGF, and the fifth is to supplement the medium with heparin. By returning non-adherent cells to the growth surface, Greenberger et al. actually teach away from the present invention, which, as set forth in claim 24 and elsewhere in the specification, comprises a method of enhancing *in vitro* proliferation of isolated human MSCs. Greenberger et al. actually teach the addition of non-MSCs to isolated MSCs, which offers no expectation of success in enhancing the proliferation of isolated MSCs. Further, Greenberger et al. list as heparin as key element of their method for growing MSCs. The present invention does not comprise the addition of heparin to the growth surface or the growth medium, and the addition of heparin would likely lead to unpredictable events, offering no expectation of success. The culture conditions, content of the conditioned media, and lack of isolated MSCs are too dissimilar from the claimed invention to offer one skilled in the art a reasonable expectation of success, and therefore, Greenberger et al., in view of Azizi et al. and Kuznetsov et al. do not render the present invention obvious.

The Examiner has cited Prockop as rendering the present invention obvious in view of Kuznetsov et al., Azizi et al. and Greenberger et al. The Examiner has argued that the molecular weight of the factors recited in claims 28 and 29 would be in the same molecular weight range of the cytokines and growth factors cited in Prockop at page 72, right hand column. Applicants argue that the molecular weights recited in claims 28 and 29 span a range of 20,000 Daltons, and the fact IL-1, IL-6, CSF-1, GM-CSF, MCSF, and c-kit ligand may or may not fall in this range does not offer a reasonable expectation of success, and does not render the present invention obvious. Even if one or all of the molecules listed in Prockop are secreted by MSCs and influence the proliferation of MSCs, the list is not exhaustive, and does not offer the skilled artisan a reasonable expectation of success in developing a conditioned medium that has the effects of the conditioned media claimed.

In addition, Prockop does not motivate or suggest methods for inducing or enhancing the proliferation of MSCs, and does not even contemplate in vitro methods for isolating or growing MSCs. Prockop is a review of the history and potential of MSCs, and does not, in any way, read on the present invention.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that claims 1-29 and 31-36 are now in condition for allowance. Applicants further submit that no new matter has been added by way of the present amendment. Reconsideration and allowance of these claims is respectfully requested at the earliest possible date.

Respectfully submitted,

Darwin J. Prockop, et al.

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(Date)

By: _____

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